UNIVERSIDADE FEDERAL DE SERGIPE
CENTRO DE CIÊNCIAS BIOLÓGICAS E DA SAUDE
DEPARTAMENTO DE FARMÁCIA

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EFEITO PROTETOR DO CARVACROL NA COLITE INDUZIDA POR ÁCIDO ACÉTICO

SÃO CRISTOVÃO

2017

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Trabalho de Conclusão de Curso apresentado ao Curso de Farmácia, da Universidade Federal de Sergipe, como requisito parcial ao título de Bacharel em Farmácia.

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SÃO CRISTOVÃO
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PROTECTIVE EFFECT OF CARVACROL ON ACETIC-ACID INDUCED COLITIS

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Abstract

The pharmacological therapy for inflammatory bowel diseases is still a challenge. Current treatment still requires new alternative options. In this study, we tested the hypothesis that carvacrol (CAR), a phenolic monoterpene with anti-inflammatory and antioxidant activities, can treat experimental colitis in mice. C57BL/6 mice (n=8/group) were submitted to intrarectal administration of acetic acid (5%) to induce colitis. Mice were pretreated with CAR (25, 50 or 100 mg/kg, p.o.) during three days (every 12 hours) before the induction. Abdominal hyperalgesia, macroscopic and microscopic colon damage, myeloperoxidase (MPO) activity, tumoral necrosis factor (TNF)-α and interleukin (IL)-1β levels, oxidative stress markers and antioxidant enzyme activities were evaluated. Pretreatment with all doses of CAR significantly decreased abdominal hyperalgesia and colon MPO activity and TNF-α and IL-1β levels. A reduction of macroscopic and microscopic damage ($p<0.05$) was observed for the doses of 50 and 100 mg/kg of CAR. Pretreatment with CAR significantly reduced lipid peroxidation (for all doses) and increased sulfhydryl groups (for 100 mg/kg). This effect was accompanied by a significant increase of catalase, superoxide dismutase and glutathione peroxidase activities. These findings indicate that CAR protected mice from acetic acid-induced colitis by reduction of inflammatory, nociceptive and oxidative damages.

Keywords: colitis; carvacrol; terpenes; inflammation; nociception; oxidative stress.

1. Introduction

Ulcerative colitis (UC) is a chronic inflammatory bowel disease that causes disrupted homeostasis and uncontrolled intestinal inflammation [1, 2]. Unlike Crohn's disease, UC is restricted to the colon and rectum [3]. The main symptoms of UC include diarrhea, abdominal cramps and recurrent blood in the stool caused by mucosal ulcers [4]. Although the etiology of UC is still not completely established, it is known that multiple factors are associated with its pathogenesis, such as genetic susceptibility, environmental and microbial factors [5] and the imbalance between immune system and intestinal microbes [6, 7]. Several studies have provided evidence that tumoral necrosis factor (TNF)-α is a key cytokine in the onset of UC, suggesting that the presence of the 308 G/A allele could confer a relative risk of 3.62 to develop UC in the general population [8].

Epidemiologic data have shown increasing global incidence and prevalence of UC in recent decades. In a military population-based study in the USA, Porter, et al. [9] found prevalence of 21.9/100 000 cases of UC per year, which in turn appeared to have a positive correlation with the elevated number of life stressors. Recently, Santos, et al. [10] showed that up to 20% of patients with UC needed to undergo colectomy due to complications of the disease in Brazil.

The current pharmacological therapy for patients with UC includes non-selective anti-inflammatory drugs and corticosteroids\immunosuppressants [11], as well as anti-TNF-α agents [12]. These drugs are used to maintain long-term remission, reduction of abnormal colonic inflammation and control of clinical symptoms, such as diarrhea, rectal bleeding and abdominal pain. However, the continuous use of these medications can cause serious side effects to patients (e.g., gastric ulcer formation, hyperglycemia, glaucoma and muscle weakness [13] and increased rates of infections, malignancies and

other adverse events [14]). Thus, a great effort has been made to develop new drugs to treat UC.

Reports from the literature indicate that the use of medicinal plants is an alternative approach to the treatment of experimental UC [15]. Medicinal plants are sources of diverse pharmacologically active compounds, like monoterpenes, which can be obtained from a number of aromatic plants, including those from the genera Lippia and Origanum [16]. Carvacrol (5-isopropyl-2-methylphenol) is a phenol monoterpene that possesses a variety of pharmacological properties, including antioxidant, antibacterial, hepatoprotective, anti-inflammatory, antinociceptive and gastroprotective activities, among others [17-20]. In this study, we evaluated the effects of carvacrol against acid acetic-induced colitis in mice.

2. Material and methods

2.1. Reagents

5-Aminosalicylic acid, 2,4-dinitrochlorobenzene, adrenaline, aprotinin A, Bradford reagent, benzomethonium chloride, bovine serum albumin, catalase, cremophor, carvacrol, dinitricbenzoic acid, glutathione reductase, glutathione, hexadecyltrimethylammonium bromide (HTAB), o-dianisidine hydrochloride, ethylenediaminetetraacetic acid (EDTA), tert-butyl hydroperoxide, horseradish peroxidase, nicotinamide adenine dinucleotide phosphate reductase (NADPH), and phenylmethylsulfonyl fluoride propionyl chloride were purchased from Sigma (St. Louis, MO, USA). Enzyme-linked immunosorbent assay (ELISA) for mouse quantitative determination of TNF-α and IL-1β was obtained from R&D Systems (Minneapolis, MN, USA). Isoflurane was obtained from Cristália (Itapira, SP, Brazil).

Acetic acid and sulfuric acid were obtained from Êxodo Científica (Hortolândia, SP, Brazil). All other reagents were obtained from Merck (São Paulo, SP, Brazil).

2.2. Animals

Male C57BL/6 mice (25-30 g) were obtained from the Animal Center of Federal University of Sergipe. They were randomly housed in appropriate cages at 22±2 °C on a 12-hour light/dark cycle (lights on from 6:00 a.m. to 6:00 p.m.), with free access to food (Purina, Brazil) and tap water. The animals were randomly distributed among the experimental groups. All experimental and euthanasia procedures were performed according to the National Laboratory Health and Use Guide (NIH) and International Council for Laboratory Animal Science (ICLAS), and were approved by the Research Ethics Committee of Federal University of Sergipe under no. 15/2014. The visual observers that carried out the nociceptive test and macroscopic and microscopic analysis were blinded from identification of the experimental groups.

2.3. Experimental design and colitis induction

Animals were divided into six groups (n = 8). Mice in group I (Vehicle + Saline) were pretreated with cremophor (0.05% prepared in 0.9% saline, as the vehicle for CAR) every 12 hours, per oral (p.o.), at 0.1 mL/10 g of body weight, during three days. In group II (Vehicle + Colitis), mice were also pretreated with cremophor in the same way. Groups III, IV and V (CAR + Colitis) were submitted to pretreatment with CAR at 25, 50 and 100 mg/kg [26,27] respectively (every 12 hours, p.o., 0.1 mL/10 g, during three days and group VI (5-ASA + Colitis) received pretreatment with 5-ASA (100 mg/kg, every 24 hours, during three days [21].

On the third day, one hour after the last pretreatment, animals from groups II to VI were submitted to colitis induction according to previous studies [22, 23], with minor modifications. Briefly, mice were submitted to isoflurane (1-2%) anesthesia and a

colon lavage was performed with 2 mL of 0.9% saline solution, with the aid of a flexible catheter (3.5 F) with approximately 3 cm in the proximal portion of the anus. Then 150 μL of 5% acetic acid solution (v/v) prepared in saline was transrectally instilled into the lumen of the colon. After administration of acetic acid, mice were maintained in a supine position for 30 s to prevent leakage of the intracolonically instilled solution. Animals from group I were submitted to the same procedure using an equal volume of saline instead of acetic acid solution. Twenty-four hours later the animals were euthanized by excess of inhaled isoflurane (5%) and colon tissue was collected for analysis.

2.4. Evaluation of colon macroscopic damage

After euthanasia, colons were immediately removed and washed in 10 mmol/L phosphate buffered saline (PBS) solution (pH 7.4). The severity of colonic lesion was evaluated using a macroscopic scale that considered the scores 0 (no macroscopic changes), 1 (only mucosal erythema), 2 (mild mucosal edema, slight hemorrhage or minor erosion), 3 (mild edema, mild ulcers, bleeding or erosions) and 4 (severe ulceration, edema and tissue necrosis), according to previous studies[22, 24].

2.5. Evaluation of inflammatory biomarkers

The myeloperoxidase (MPO) activity was measured using the technique described by Bradley, et al. [25] with minor modifications. Colon samples (~ 100 mg) were collected, weighed, cut into small pieces and homogenized in potassium phosphate buffer (50 mmol/L, pH 6.0, containing 0.5% HTAB). Then 1-mL aliquots of the homogenate were centrifuged (10 min, 18,000 g, 4 °C) to obtain the supernatant. In a 96-well plate, 20 μL of the supernatant was pipetted and 200 μL of a solution containing o-dianisidine dihydrochloride (0.167 mg/mL, prepared in 50 mmol/L of potassium

phosphate buffer containing 0.005% of H_2O_2). The results were expressed as units of MPO per mg of tissue (UMPO/mg tissue).

The levels of TNF-α and IL-1β were quantified in colon tissues through enzyme immunoassay (ELISA) according to the manufacturer's instructions. For this determination, colons were removed and homogenized in PBS (in mmol/L NaCl 137; KCl 2.7; Na_2HPO_4 8.1; KH_2PO_4 1.5, pH 7.2) containing Tween 20 (0.05%), PMSF (0.1 mmol/L), benzomethonium chloride (0.1 mmol/L), EDTA (10 mmol/L) and aprotinin A (2 ng/mL). The amount of protein in each sample was measured using the Bradford method and the results were expressed in pg of cytokine/mg of protein.

2.6. Histological analysis

For the histological evaluation, colons were removed and fixed with 10% buffered formalin. These samples were embedded in paraffin, following routine histological processing, and cut into 5 µm sections and stained with hematoxylin and eosin. The presence of architecture loss, cellular infiltrate mucosa, muscle submucosa thickening, abscess formation in crypts/hemorrhage and depletion of goblet cells was assessed by a qualitative score that considered: 0 (no detectable changes), 1 (mild injury), 2 (moderate injury), and 3 (severe injury) [26]. Qualitative analysis was performed on 400× magnified images.

2.7. Measurement of abdominal hyperalgesia

The mechanical abdominal hyperalgesia was evaluated with the electronic von Frey test[27]. Animals were acclimated for 30 minutes before the test in individual cages and mechanical stimuli were applied in the abdomen of each animal. The first measurement was performed before colitis induction by acetic acid (basal) and the second measurement was performed 23 hours after induction.

For each measurement, the electronic von Frey tip was applied as a stimulus of increasing pressure in the lower abdominal region until the mice presented a withdrawal response. The force required to cause this response was considered the threshold value. This procedure was performed in triplicate and the mean was used for each animal. The test was performed in a covert manner, so that the examiner who carried out the measurements had no knowledge about the experimental groups.

The variation (Δ) of stimulus intensity (expressed in grams) was calculated by subtracting the values obtained after induction from the basal values.

2.8. Evaluation of oxidative stress and antioxidant enzyme activity

Samples of colon were homogenized in potassium phosphate buffer (50 mmol/L, pH 7.4, 1 g of tissue/5 mL of buffer). The amount of protein in each sample was measured using the Bradford method.

The determination of the thiobarbituric acid reaction products was performed according to a previous description [28]. Briefly, the homogenate (100 μL) was added with 950 μL of the acid solution (20% acetic acid and 0.5% thiobarbituric acid), agitated and incubated at 85 °C for 60 min. After incubation, samples were centrifuged for 5 min at 18,000 g. The supernatant absorbance was read at 535 nm in a microplate reader and corrected by the reading at 572 nm. The results were calculated using the molar extinction coefficient of 1.55×10^{5} $mol^{-1}.L.cm^{-1}$ and expressed as nmols of malondialdehyde (MDA) per mg of tissue.

The sulfhydryl groups content of the proteins was measured as previously described [29]. Colon homogenate samples (50 μg of proteins in 50 μL) were reacted with 10 μL of dinitricbenzoic acid for 1 hour and the absorbance was read at 412 nm. Results were expressed as SH (μmol/mg of protein).

The catalase (CAT) activity was determined as previously described [30]. The homogenate (300 µL) was incubated with 500 µL of H_2O_2 (10 mmol/L) and after 5 min the reaction was stopped with 500 µL of 1 mmol/L of NaN_3 and 200 µL of HRP reagent was added. The absorbance was detected at 460 nm and the results were plotted against an H_2O_2 curve and expressed as units of CAT/mg of protein.

The analysis of superoxide dismutase (SOD) was based on the inhibition of the reaction of the superoxide radical with adrenaline [31]. The SOD reaction was performed as a protein curve for each sample (7.5 µg, 15 µg and 30 µg of protein), which was incubated in the presence of catalase (5 µL, 10 µmoL/L) and then adrenaline (5 µL, 60 mmol/L). The kinetic reading was performed during 10 min at 480 nm at a temperature of 32 °C and the values were expressed as units of SOD/mg protein.

The activity of glutathione peroxidase (GSH-Px) was determined according to the method described by Paglia and Valentine [32]. For this assay, 30 µL of homogenate was added to 5 µL of GSH (0.08 mol/L), 5 µL of GR (9.6 U/mL), 145 µL of phosphate buffer (50 mmol/L) and EDTA (0.1 mol/L) and incubated for 5 min at 37 °C. Then, 5 µL of tert-butyl hydroperoxide (0.3%) and 10 µL of NADPH (1.2 mmol/L) were added and the assay was monitored at 340 nm for 10 min at 37 °C. The results were expressed as µmol of GSH/min/mg of protein.

The determination of glutathione S-transferase (GST) was performed according to a previous study [33]. In this assay, 10 µL of homogenate is added to 5 µL of 1-chloro-2,4-dinitrobenzene (0.1 mol/L) following incubation for 10 min and addition of 5 µL of GSH (0.1 mol/L). The absorbance was monitored for 15 min (25 °C, 340 nm). The results were expressed in µmol of GSH conjugate/min/mg of protein.

2.9. Statistical analysis

The results were expressed as mean ± S.E.M and data were analyzed by using the GraphPad Prism software (San Diego, CA, USA) version 5.0. Parametric data were analyzed by one-way analysis of variance (ANOVA) followed by the Tukey test. Nonparametric data (macroscopic and microscopic scores) were analyzed by the Kruskal-Wallis test followed by Dunn's test. Differences were considered significant if $p<0.05$.

3. Results and Discussion

3.1. Pretreatment with CAR reduces colon inflammation

Treatment with CAR (50 and 100 mg/kg), performed three days before colitis induction, reduced ($p<0.05$ for 50 mg/kg and $p<0.01$ for 100 mg/kg) the macroscopic damage (mucosal edema, ulceration, hemorrhage and/or erosions) induced by acetic acid (5%), when compared to animals pretreated with vehicle (Figure 1A). Although this effect was significantly observed from the dose of 50 mg/kg, pretreatment with all doses reduced MPO activity ($p<0.001$), as did pretreatment with the positive control drug, 5-ASA (100 mg/kg; $p<0.01$, Figure 1B). These results suggest that CAR protected mice from the acute injury caused by acetic acid and that inhibition of neutrophil infiltration is an event related to this beneficial effect. Since neutrophils are strongly modulated by cytokines like TNF-α and IL-1β [34], we measured the concentration of these mediators in the colon tissue and observed that the pretreatment with CAR consistently inhibited TNF-α ($p<0.001$ for all doses) and IL-1β levels ($p<0.01$ for 25 mg/kg and $p<0.001$ for 50 and 100 mg/kg). A significant reduction of TNF-α, but not IL-1β, was also achieved by pretreatment with 5-ASA (Figures 1C and D). These results are in agreement with previous studies that showed that CAR (25-100 mg/kg) attenuated the neutrophil influx and TNF-α production during pleurisy induced by

carrageenan in mice [35], reduced the TNF-α and IL-1β mRNA expression and production in mice paws injected with complex Freund adjuvant [36], and decreased the MPO activity and cytokine levels in irinotecan-induced intestinal mucositis [37].

Corroborating these results, the histological analysis of colon tissues revealed that the alterations induced by intrarectal acetic acid administration (loss of tissue architecture, cellular infiltrate in the mucosa, submucosa muscle thickening, abscess formation and hemorrhage in crypts and depletion of goblet cells) were less observed in animals treated with all doses of CAR or 5-ASA (Figures 2A-F). These findings were confirmed by the lower total histopathological scores of colon slices from mice pretreated with CAR ($p<0.05$), when compared to the vehicle group. This effect was also observed for 5-ASA ($p<0.05$) (Figure 2G).

3.2. Pretreatment with CAR reduces abdominal hyperalgesia

As a consequence of colitis induction, mice developed abdominal hyperalgesia. This effect was measured 23 hours after colitis induction as the decreased intensity of mechanical stimulus needed to cause withdrawal behavior in mice injected with acetic acid ($p<0.001$, Figure 3), compared with animals injected with saline. This result agrees with previous descriptions of alterations in the visceral sensitivity in rats submitted to acetic acid-induced colitis [38]. Visceral pain was also described in the model of colitis induced by 2,4,6-trinitrobenzene sulfonic acid (TNBS) in rats [39]. Besides this, visceral pain is one of the main complaints of patients with UC [40], reinforcing the translational potential of evaluating abdominal hyperalgesia in experimental colitis.

Interestingly, the pretreatment with CAR produced an inhibitory effect ($p<0.05$ for 25 and 100 mg/kg and $p<0.001$ for 50 mg/kg) on the mechanical abdominal hyperalgesia provoked by acetic acid-induced colitis, as did 5-ASA ($p<0.001$, Figure 3).

Further studies have shown that CAR causes antinociceptive effects in mice. Guimaraes, et al. [35] showed that pretreatment of mice with CAR (25-100 mg/kg, i.p.) reduced acetic acid-induced abdominal writhing, decreased formalin, glutamate or capsaicin-induced paw licking and increased latency in the hot plate test. Another study also demonstrated that CAR (50 or 100 mg/kg, i.p.) decreased the mechanical hyperalgesia caused by carrageenan, TNF-α, prostaglandin E_2 and dopamine injection in mice paw [41]. Treatment with CAR (50 mg/kg, s.c.) also reduced mechanical hyperalgesia and spontaneous or palpation-induced nociception in mice with cancer pain. This effect was associated with reduced cFos positive neurons in lamina I of spinal cord and increased cFos positive neurons in descending inhibitory pain pathway structures, confirming the modulation of central nociceptive mechanisms by CAR [41].

Altogether, these studies have suggested the possibility that the anti-hyperalgesic effect of CAR observed in our study might be a consequence of decreased colon inflammation or modulation of the central nervous system.

A mechanistic possibility for CAR actions is the activation of the transient receptor potential A1 (TRPA1) [42]. Intriguingly, this receptor is involved in the mechanical hypersensitivity of the colon through direct activation of sensory fibers, leading to the production of substance P and calcitonin gene-related peptide Bautista, et al. [43]. The exact role of TRPA1 receptors in colitis is still controversial, since substance P is essential for the development of colitis by TNBS or dextran sulphate sodium [44], but CGRP protected mice from colon damage by TNBS [45]. Nevertheless, a previous study demonstrated that cannabichromene, a TRPA1 agonist, reduced colitis induced by dinitrobenzene sulphonic acid in mice [46]. Of interest, capsazepine, a well-known TRPV1 antagonist, was recently described as an activator of TRPA1 and the topical administration (enema) of capsazepine to mouse colons initially

led to a nociceptive response, but this response was susceptible to desensitization after 4 days of administration [47]. This study supports the possibility that the reversion of hyperalgesia by CAR in the model of acetic acid-induced colitis could account for the desensitization of the $TRPA_1$ receptor.

3.3. Modulation of oxidative stress and antioxidant enzymes by CAR

It is well known that CAR possesses antioxidant activity in vitro [48] and in vivo [49-51]. The possibility that the oxidative stress plays a role in the effect of CAR was investigated through measurement of oxidative stress markers and antioxidant enzyme activity during colitis induced by acetic acid. Figure 4A demonstrates that the lipid peroxidation observed in inflamed colon was lower in mice treated with CAR ($p<0.01$ for 25 mg/kg and $p<0.001$ for 50 and 100 mg/kg) or 5-ASA ($p<0.001$) when compared to the vehicle + colitis group. Also, colon inflammation by acetic acid decreased the sulfhydryl groups content in colon ($p<0.001$) and the pretreatment with CAR and 5-ASA, at a dose 100 mg/kg, reversed this oxidative effect ($p<0.05$) when compared to the vehicle + colitis group (Figure 4B).

Since these antioxidant effects observed of CAR in the colonic tissue could occur due to modulation of antioxidant enzymes, the activities of CAT, SOD, GPx and GST were evaluated. Figure 5(A-D) shows that the induction of colitis decreased the activities of these enzymes ($p<0.001$) when compared to the vehicle + saline group. Pretreatment with CAR partially reverted the deleterious effect of acetic acid on the activity of CAT ($p<0.05$ for 25 and 100 mg/kg), SOD ($p<0.01$ for 100 mg/kg) and GPx ($p<0.05$ for all doses), without affecting the activity of GST, in comparison to the vehicle plus colitis group (Figure 5). The administration of 5-ASA also restored the activity of CAT ($p<0.001$), SOD ($p<0.001$), GPx ($p<0.001$) and GST ($p<0.05$), when compared with the vehicle + colitis group (Figure 5).

These results suggest that the mechanisms underlying the protective effects of CAR on acetic acid-induced colitis can account for the reduction of oxidative stress in the mouse colon. Although this association is reasonable, it should be considered that the decrease in oxidative stress could be caused by the lower inflammatory damage to the colon. In any event, a striking feature of colitis induced by acetic acid or other agents is the presence of oxidative damage, which plays a critical role in the initiation and progression of colitis [52-54]. Thus, excessive reactive species causes colon damage and contributes to the formation of secondary messengers that activate intracellular signaling pathways and exacerbate inflammation, leading to widespread effects [55]. Corroborating our findings, previous studies have reported the antioxidant effect of CAR in the models of colitis-associated colon cancer in rats [49], irinotecan-induced intestinal mucositis in mice [37], liver damage in elderly rats [50] and doxorubicin-induced cardiotoxicity in rats [51].

In summary, CAR attenuated acetic acid-induced colitis in mice through anti-inflammatory mechanisms by inhibiting TNF-α and IL-1β and reducing oxidative damage with the participation of antioxidant enzymes, which collectively led to reduction of abdominal hyperalgesia. These results demonstrate the potential of CAR for the treatment of colitis.

Acknowledgments

We thank “Conselho Nacional de Pesquisa e Desenvolvimento Científico” (CNPq) and “Coordenação de Aperfeiçoamento de Pessoal de Nível Superior” (CAPES) for financial support. EAC and LJQJ are beneficiaries of CNPq productivity grants.

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ANEXOS

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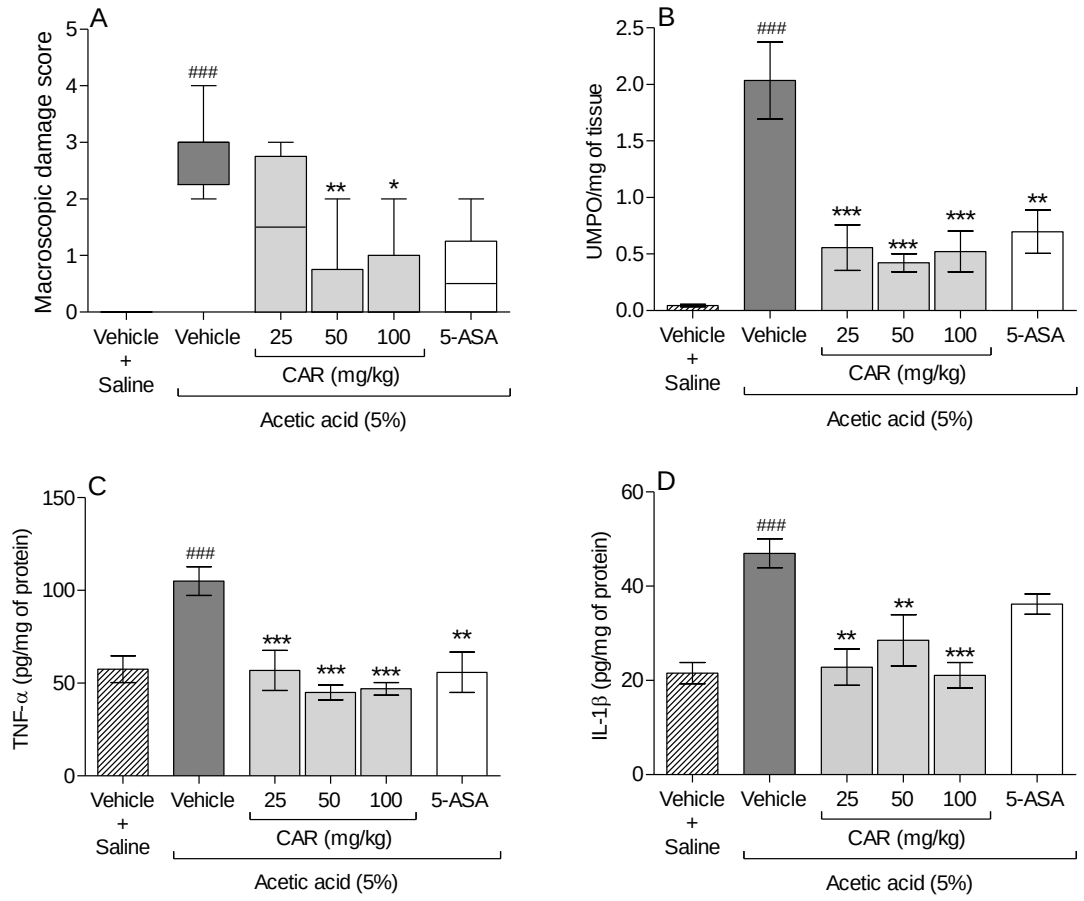

Figure 1. **Pretreatment with carvacrol (CAR: 25, 50, or 100 mg/kg) reduces colon inflammation induced by acetic acid.** Animals (n=8) were pretreated with CAR (p.o.) or 5-aminosalicylic acid (5-ASA; 100 mg/kg, p.o.) and colitis was induced by intracolonic injection of acetic acid (5%). Panel A shows the box-and-whisker plots (minimum to maximum) for the macroscopic score for colonic damage. Panels B, C and D demonstrate the mean±SEM for the units of myeloperoxidase activity (UMPO)/mg of colonic and the levels of tumor necrosis factor-alpha (TNF-α) and interleukin-1β (IL-1β), respectively. $^{###}p<0.001$ vs. vehicle + saline group; $^{**}p<0.01$ or $^{***}p<0.001$ vs.

vehicle + acetic acid group (Panel A: Kruskal-Wallis followed by Dunn's test; panels B, C and D: one-way ANOVA followed by Tukey test).

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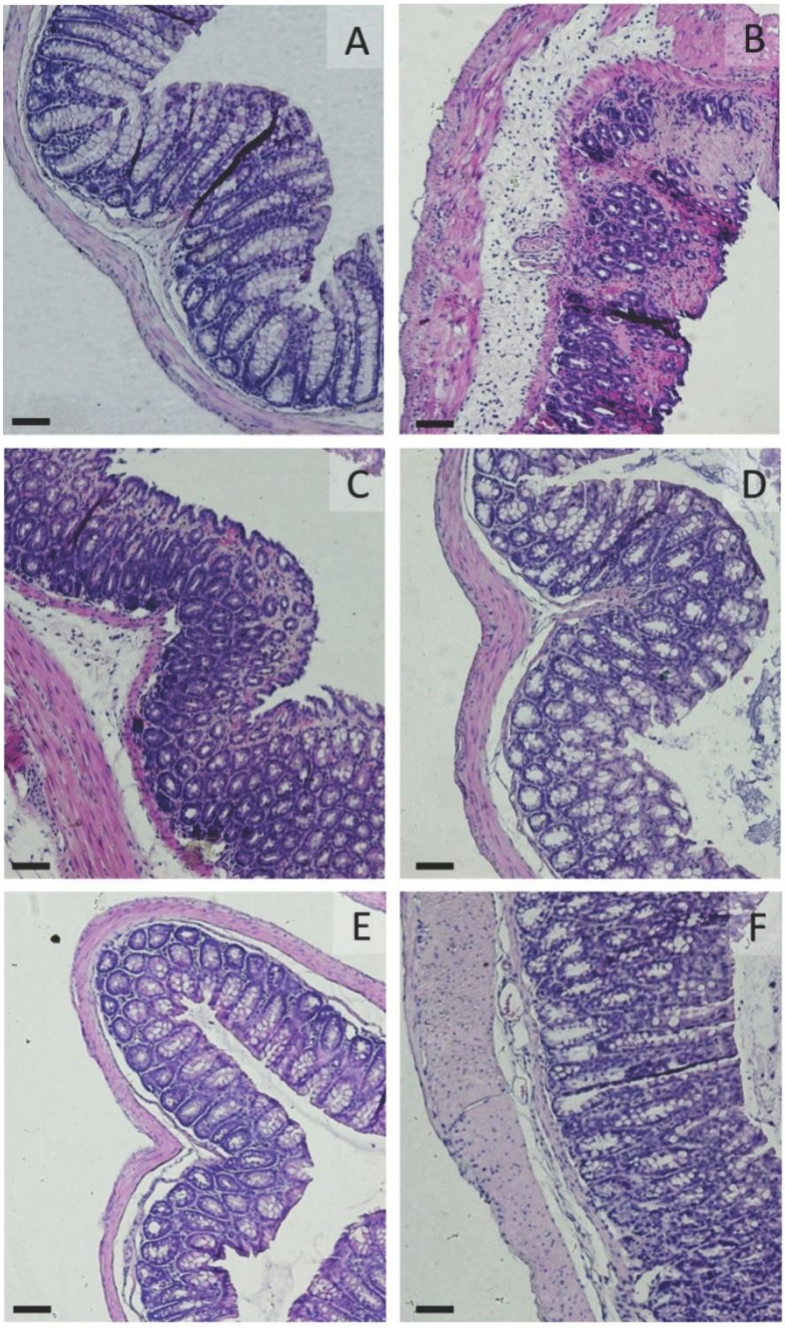


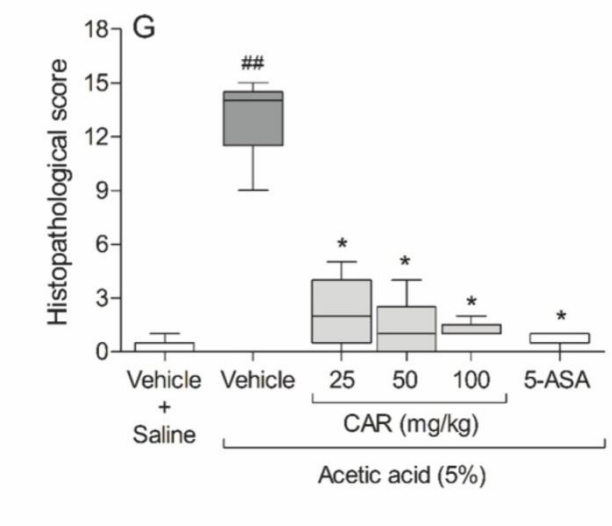


Figure 2. Pretreatment with carvacrol reduces histological alterations induced by acetic acid 5% in mice colon. Images were captured at 10-fold magnification of colon tissue slices stained with hematoxylin and eosin. Normal structure of colon tissue is demonstrated in the vehicle + saline group (Panel A). Panel B shows the loss of integrity of colon architecture in animals from vehicle + acetic acid group. Panels C, D and E represent the tissues of animals pretreated with carvacrol (25, 50 and 100 mg/kg, respectively) and Panel F represents the tissue of mice pretreated with 5-ASA (100 mg/kg). The sum of histopathological scores is represented in Panel G as a box-and whisker plot (minimum to maximum). $^{\#\#}p<0.01$ vs. vehicle + saline group; $^{*}p<0.05$ vs. vehicle + acetic acid group (Kruskal-Wallis followed by Dunn's test, n=4).

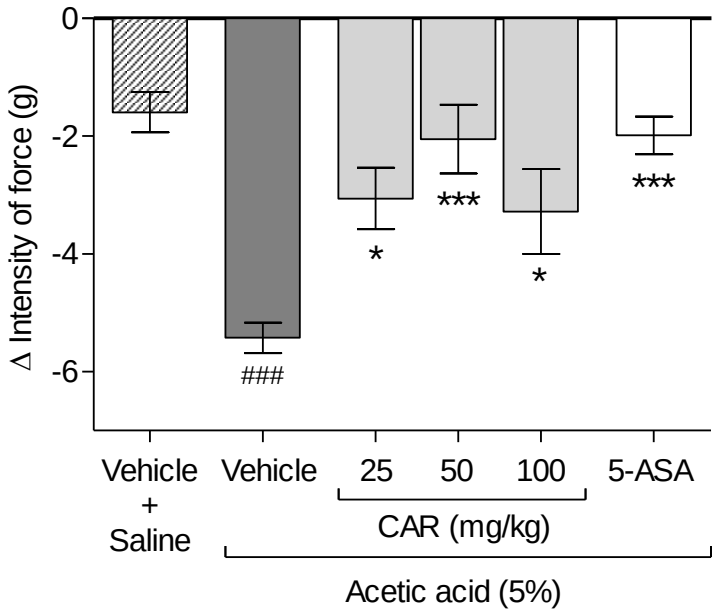


Figure 3. **Pretreatment with carvacrol (CAR: 25, 50, or 100 mg/kg) decreases abdominal hyperalgesia in mice submitted to acetic acid-induced colitis.** Animals (n=8) were pretreated with CAR (p.o.) or 5-aminosalicylic acid (5-ASA; 100 mg/kg,

p.o.) and colitis was induced by intracolonic injection of acetic acid (5%). Data are shown as mean±SEM (n=8) of the variation (Δ) of the withdrawal threshold (in grams) to tactile stimulation of the abdomen before and after xx hours of induction of colitis. $^{###}p<0.001$ vs saline group; $^{*}p<0.05$ or $^{***}p<0.001$ vs. vehicle + acetic acid group (one-way ANOVA followed by Tukey test).

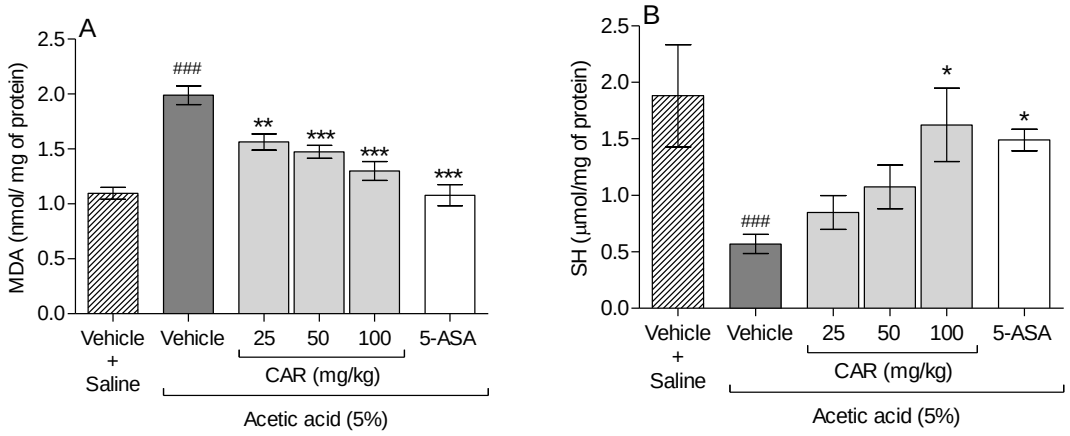


Figure 4. Pretreatment with carvacrol (CAR) reduces oxidative stress in colon tissue. Animals (n=8) were pretreated with CAR (p.o.) or 5-aminosalicylic acid (5-ASA; 100 mg/kg, p.o.) and colitis was induced by intracolonic injection of acetic acid (5%). Lipoperoxidation, measured as the concentration of malondialdehyde (MDA; Panel A) and sulfhydryl groups (SH; panel B) were determined in colon tissue. Data are expressed as mean±SEM for n=8. $^{###}p<0.001$ vs. vehicle+saline group and $^{*}p<0.05$; $^{**}p<0.01$ or $^{***}p<0.001$ vs. vehicle+acetic acid group (one-way ANOVA followed by Tukey test).

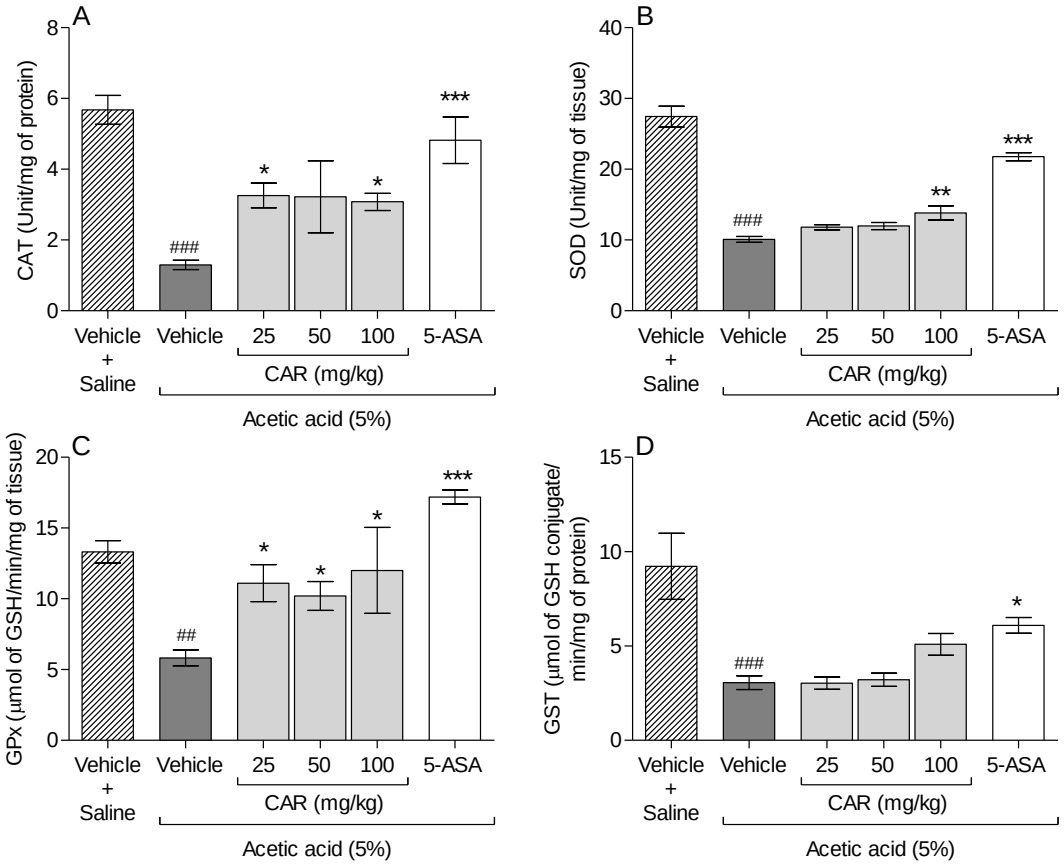
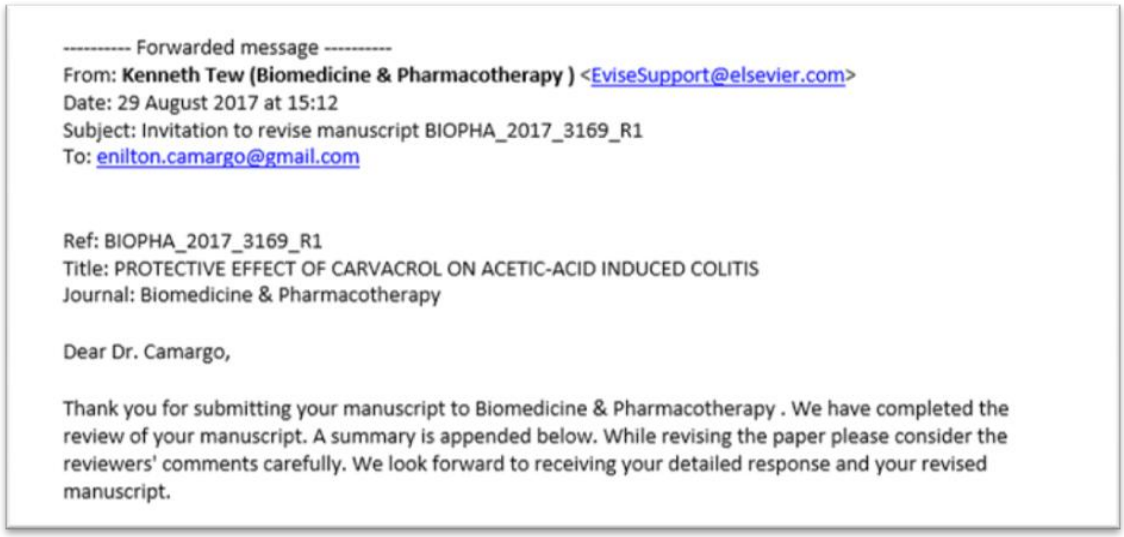


Figure 5. Modulation of antioxidant enzymes activity by pretreatment with carvacrol. Animals (n=8) were pretreated with CAR (p.o.) or 5-aminosalicylic acid (5-ASA; 100 mg/kg, p.o.) and colitis was induced by intracolonic injection of acetic acid (5%). Catalase (CAT, Panel A), superoxide dismutase (SOD, Panel B), glutathione (GSH) peroxidase (Panel C) and GSH transferase (Panel D) activities were measured in colon tissue of mice. Data are expressed as mean±SEM for n=8. $^{\#\#}p<0.01$ or $^{\#\#\#}p<0.001$ vs. vehicle+saline group and $^{*}p<0.05$; $^{**}p<0.01$ or $^{***}p<0.001$ vs. vehicle+acetic acid group (one-way ANOVA followed by Tukey test).

Documents:



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Title: PROTECTIVE EFFECT OF CARVACROL ON ACETIC-ACID INDUCED COLITIS
Journal: Biomedicine & Pharmacotherapy

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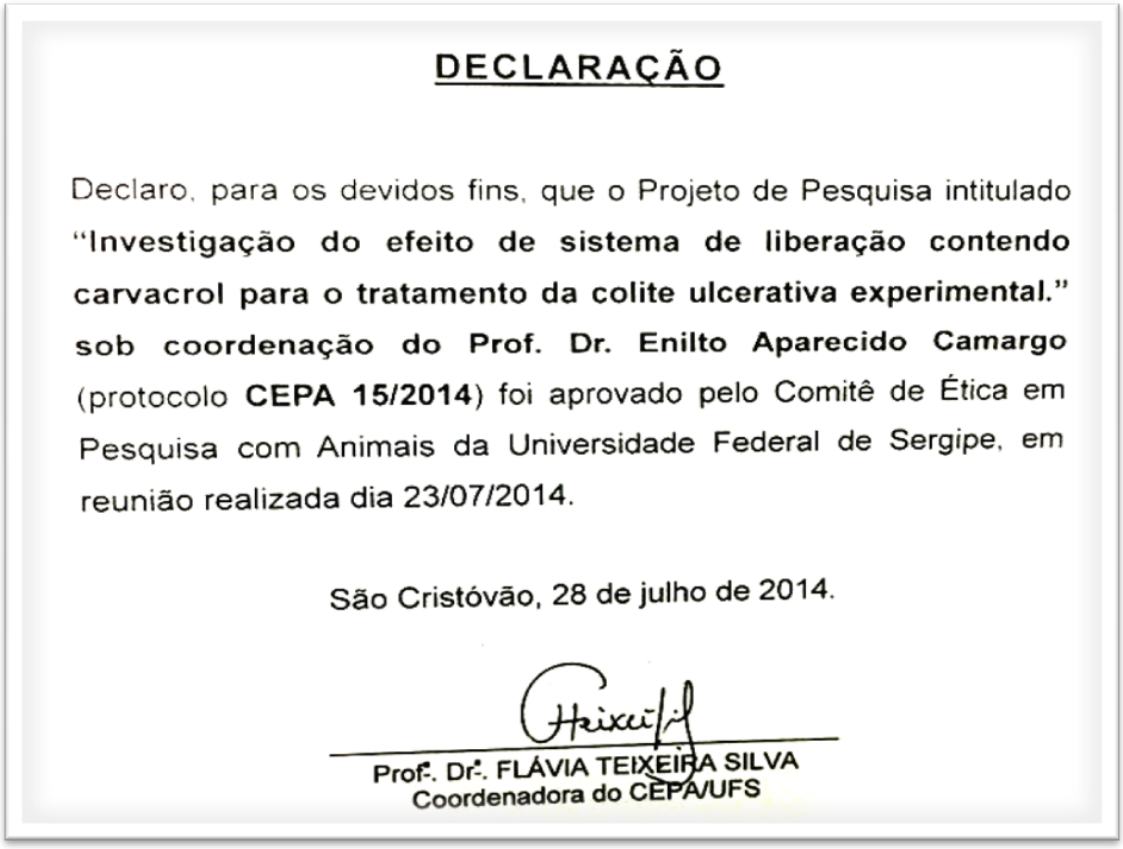
Figura 1. Comprovante de Submissão de Artigo



DECLARAÇÃO

Declaro, para os devidos fins, que o Projeto de Pesquisa intitulado "**Investigação do efeito de sistema de liberação contendo carvacrol para o tratamento da colite ulcerativa experimental.**" **sob coordenação do Prof. Dr. Enilto Aparecido Camargo** (protocolo **CEPA 15/2014**) foi aprovado pelo Comitê de Ética em Pesquisa com Animais da Universidade Federal de Sergipe, em reunião realizada dia 23/07/2014.

São Cristóvão, 28 de julho de 2014.

Profª. Drª. FLÁVIA TEIXEIRA SILVA
Coordenadora do CEPA/UFS

Figura 2. Declaração de Submissão do CEPA